

CHARACTERIZATION OF PECTIN METHYLESTERASE FROM MARVEL TOMATOES

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ABSTRACT

Pectin methylesterase isolated from Marvel tomatoes exhibited maximum activity at pH 7,5 in 0,15 M NaCl and 1 % citrus pectin and has a K_m of 0,1 % for citrus pectin. The enzyme revealed considerable heat-stability partly ascribed to the finding that it contains carbohydrate components consisting of arabinose, xylose, mannose, galactose and glucose. The presence of 18 kinds of amino acids was established in the enzyme.

UITTREKSEL

KARAKTERISERING VAN PEKTIENMETIELESTERASE UIT MARVEL TAMATIES

Pektiemetielesterase uit Marvel-tamaties het optimum aktiwiteit by pH 7,5 in 0,15 M NaCl en 1 % sitruspektien getoon en het 'n K_m -waarde van 0,1 %. Die ensiem het 'n hoë mate van hitte-stabiliteit getoon wat moontlik toegeskryf kan word aan die feit dat dit 'n koolhidraatgedeelte bevat. Gaschromatografiese-analises van die koolhidraatgedeelte het die teenwoordigheid van arabinose, xilose, mannose, galaktose en glukose aangetoon. Dit is ook vasgestel dat 18 soorte aminosure in die ensiem teenwoordig is.

INTRODUCTION

The isolation and purification of pectin methylesterase (PME) (E.C. 3.1.1.11.) from Marvel tomatoes has been described in a previous paper (Theron, De Villiers & Schmidt, 1977). The enzyme was extracted with NaCl and purification was achieved by means of ammonium sulphate fractionation and chromatography on various Sephadex G-types. It was shown that the purified enzyme had an apparent lack of a net charge and that the enzyme contained a carbohydrate residue. In the present paper more properties of the enzyme, as well as the chemical composition of the protein and carbohydrate residue of the enzyme, are described.

MATERIAL AND METHODS

Plant material

Mature fruits of tomatoes (*Lycopersicum esculentum*, cv. Marvel) were purchased from a local dealer and stored at -10°C until used.

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Substrate

A commercial preparation of citrus pectin (100 grade, 24 % esterified) was used throughout the investigation. A 1 % solution in 0,15 M NaCl (pH 7,5) was prepared daily.

Isolation and purification

PME was isolated and purified as described previously (Theron, De Villiers & Schmidt, 1977).

Determination of PME activity

Determination of PME activity was carried out according to the method of MacDonnel, Jansen & Lineweaver (1945) by means of continuous titration of the carboxyl groups liberated during the course of the reaction.

Effect of enzyme concentration

To study the effect of PME concentration on the initial reaction velocity 20 cm³ of substrate was used. Enzyme activity was determined in 5, 10 and 15 cm³ of the enzyme solution (in 0,15 M NaCl) respectively. The volume and concentration of the substrate for each determination was adjusted to maintain a constant final concentration and volume.

Effect of substrate concentration

For the determination of the K_m -value of tomato PME the same assay procedure as described above was employed at pH 7,5 except that the citrus pectin concentration was varied. The enzyme concentrations used were chosen to effect a convenient change of pH per min. As the molecular mass of the substrate is not known, the K_m -value is presented as g/100 cm³ (%) according to Nakagawa, Yanagawa & Takehana (1970).

Effect of pH

The determination of activity at various pH values was carried out according to the method of Nakagawa, Yanagawa & Takehana (1970). The reaction was carried out at 30 °C for 10 min, recording alkali consumption every min.

Heat stability

To study the effect of temperature on the stability of PME, enzyme preparations were kept at the various temperatures in a water bath for exactly 5 min, immediately cooled to 30 °C and added to the substrate. PME activity was determined as described above.

Amino acid composition

Samples of purified PME were hydrolysed in sealed evacuated tubes with 1 cm³ 6 M HCl at 110 °C for 22 hours. After removal of the HCl by evaporation under reduced pressure, the residues were dissolved in citrate buffer at pH 2,2 and

the amino acid composition of the hydrolysates determined with a Beckman model 120 C automatic amino acid analyser.

Carbohydrate composition

For the determination of the carbohydrate composition of purified PME, the methods of Clamp, Dawson & Hough (1967) and Chambers, Bhatti & Clamp (1970) were used.

Trimethylsilyl (TMS) derivatives of the carbohydrate components were separated with a Becker-Packard model 419 gas chromatograph fitted with a column packed with OVI on chromosorb WAW (80/100 mesh).

Determination of protein

Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as standard.

RESULTS

1. Effect of enzyme concentration

As shown in Fig. 1 the initial reaction velocity of 0.02 M NaOH consumption was proportional to enzyme concentration (expressed as cm^3 enzyme solution).

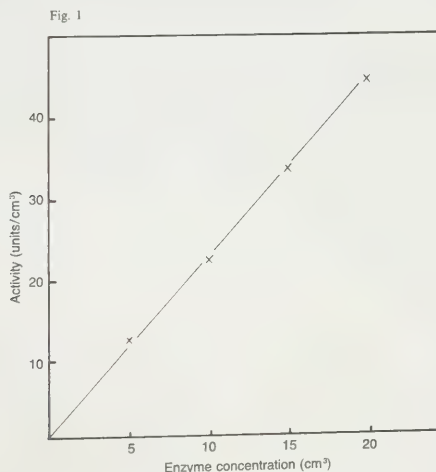


FIG. 1.

The effect of enzyme concentration on the reaction velocity of PME from Marvel tomatoes

2. Effect of pH

The effect of pH on the activity of PME shows the typical bell-shaped profile of enzyme activity against pH with an optimum at pH 7.5 (Fig. 2).

Fig. 2

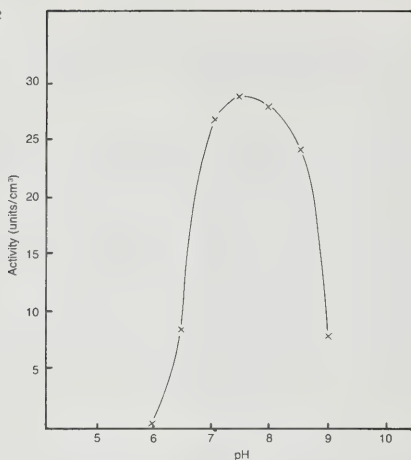


FIG. 2.

The effect of pH on the activity of PME from Marvel tomatoes.

3. Heat stability

The temperature profile of tomato PME in 0,15 M NaCl is illustrated in Fig. 3. It is evident that approximately 80 % activity remained after incubation at 50 °C for 5 min while only about 30 % residual activity remained after incubation at 60 °C. In spite of the initial heat resistance, the enzyme was completely inactivated at 70 °C for 5 min.

Fig. 3

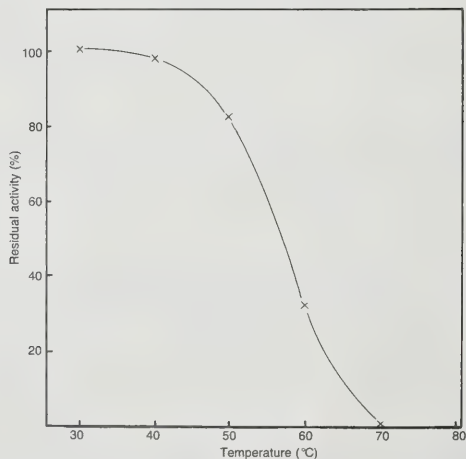


FIG. 3.

The effect of temperature on the activity of PME from Marvel tomatoes.

4. Effect of substrate

Fig. 4 shows the conventional double reciprocal plot of different pectin concentrations on the activity of the enzyme. A K_m -value of 0,1 % was obtained.

Fig. 4

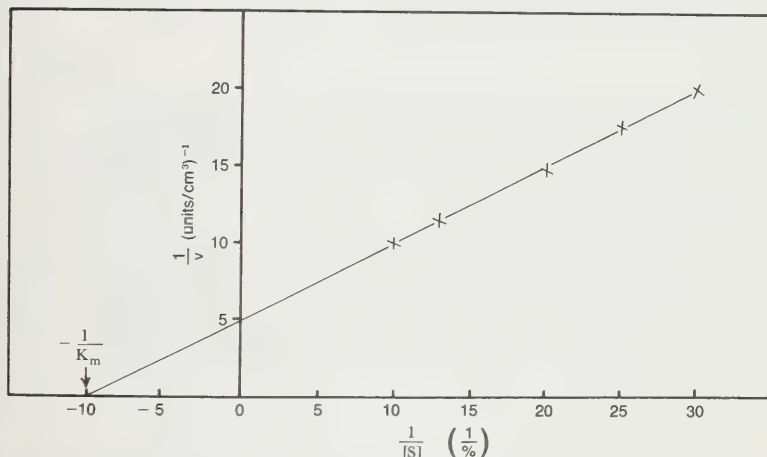


FIG. 4.

Lineweaver-Burk presentation of the effect of substrate concentration on the activity of PME from Marvel tomatoes.

TABLE 1.

Amino acid composition of purified PME from Marvel tomatoes

Components	Amino acid content (mg/100 mg)
Lysine	5,36
Histidine	0,93
Ammonia	4,08
Arginine	3,09
Asparagine/Aspartic Acid	9,51
Threonine	5,71
Serine	5,15
Glutamine/Glutamic Acid	6,91
Proline	4,40
Glycine	4,86
Alanine	4,91
Cysteine	*
Valine	3,39
Methionine	*
Isoleucine	3,33
Leucine	5,17
Tyrosine	2,89
Phenylalanine	2,72

*Trace amounts

5. Amino acid composition

Table 1 shows the amino acid composition of tomato PME. The presence of eighteen kinds of amino acids, with asparagine/aspartic acid and glutamine/glutamic acid present in the highest concentrations, were established in the enzyme. Tryptophan was not determined while only trace amounts of cysteine and methionine were found in the enzyme.

6. Carbohydrate composition

The carbohydrate composition of the enzyme is presented in Table 2. From this it is clear that five sugars are present in the enzyme with mannose and glucose present in the highest concentration.

TABLE 2.
Monosaccharides identified in the carbohydrate moiety of purified PME from Marvel tomatoes

Components	Carbohydrate content (mg/100 mg)
Arabinose	3,3
Xylose	2,0
Galactose	0,7
Mannose	6,8
Glucose	4,2

DISCUSSION

Some properties of PME from Marvel tomatoes are described in the present paper. The enzyme showed maximum activity at pH 7,5 compared to an optimum pH of 8,0 obtained by Nakagawa *et al.* (1970) in the Hikari variety of tomatoes. The K_m -value for citrus pectin was 0,1 % in comparison to that of 0,24 % found by Nakagawa *et al.* (1970).

In a previous paper we reported that PME contains carbohydrate (Theron *et al.*, 1977). Gas chromatographic analysis of the purified enzyme confirmed this, indicating the presence of five sugars in the enzyme. These results, suggesting a possible glycoprotein nature of the enzyme as was suggested by Delincée and Radola (1970), may probably be the reason for the apparent lack of a net charge on the enzyme (Theron *et al.*, 1977).

Similar to the results obtained by Nakagawa *et al.* (1970) the present study also revealed that PME from Marvel tomatoes is relatively heat stable. The presence of 18 kinds of amino acids were established in the purified enzyme. It was found that the amino acid composition of PME in the present study is very similar to that found by Nakagawa *et al.* (1970) in the Hikari variety of tomatoes except that the enzyme in the present study contains a small amount of histidine

and only trace amounts of cysteine and methionine since these amino acids were degraded during acid hydrolysis.

It is generally accepted that the intercellular cement of fruit cellwalls is attached by various pectic enzymes with the resultant loss of rigidity of the cells. However, the precise role of PME in such a function has not yet been established.

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